REMARKS

I. Status of the Application

Claims 1-46 are presently pending in the application. Applicants gratefully acknowledge the Examiner's withdrawal of claims 39-44 under 35 U.S.C. § 112, second paragraph, as being indefinite. Claims 1-3, 5, 9 and 9-31 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Jena et al., *J. Immunol. Methods* (1996) 190:199, in view of Lockhart et al., WO 97/10365. Claims 4, 6-8, 41, 45 and 46 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Jena et al. in view of Lockhart et al., further in view of Hampson et al., U.S. Patent No. 6,066,457. Claims 32-44 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Jena et al. in view of Lockhart et al., further in view of Church et al., U.S. Patent No. 6,511,803.

Applicants have amended the claims to more clearly define and distinctly characterize Applicants' novel invention. Support for the amendments can be found in the specification and the claims as originally filed. Specifically, support for the amendments to claims 1, 32, 39, 40, 42 and 43 to recite linearly amplifying can be found in the specification at least at page 13, lines 10-12, where Applicants teach "methods also provide for linear amplification...".

Applicants respectfully request entry and consideration of the foregoing remarks, which are intended to place this case in condition for allowance. Applicants respectfully submit that the amendments presented herein do not raise new issues requiring further search. The present Amendment and Response is being filed within 2 months of the mailing date of the Final Office Action, accordingly, Applicants request issuance of an advisory action.

II. Claims 1-3, 5 and 9-31 Are Patentable Over Jena et al. in View of Lockhart et al.

At page 2, paragraph 5 of the instant Office Action, claims 1-3, 5 and 9-31 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Jena et al., *J. Immunol. Methods* (1996) 190:199, in view of Lockhart et al., WO 97/10365. The Examiner asserts that the phrase "amplifying the population" embraces any number of rounds of amplification cycles and not just a single round of amplification as Applicants contend. The Examiner further asserts that Lockhart et al. employs a microarray comprising a plurality of immobilized probes, which would necessarily determine at least two genes when employed for the method of Jena et al. Applicants respectfully traverse this rejection.

Applicants' amended claims are directed to novel methods of monitoring gene expression wherein RNA is obtained from a sample comprising fewer than 1000 cells or from a single cell, a first population of cDNA is generated from the RNA, the first population of cDNA is ***linearly amplified*** to produce a linearly amplified second population of cDNA, the linearly amplified second population of cDNA is labeled with a detectable label, and an array of probes is contacted with the linearly amplified population of labeled cDNA to determine relative expression of at least two genes.

Applicants' claimed invention provides for ***linear amplification***, which preserves the proportional levels of cDNA species in a cDNA population relative to proportional levels of RNA species in the RNA population from which the cDNA was generated (page 13, lines 10-12). Applicants' novel method allows one of skill in the art to gauge relative expression of a variety of genes in a cell including genes having very low levels of expression and abundantly expressed genes. Applicants have demonstrated that the claimed invention provides expression profiles that correlate with actual relative gene expression in the cell, and represent genes having

low levels of expression (see page 32, line 31 to page 35, line 2 of the specification; Tietjen et al. (2003) *Neuron* 38:161, given to Examiner Siew in the interview on January 20, 2004). Applicants' invention is an advance over methods in the art which lead to ***disproportionate*** amplification of abundant transcripts relative to rare transcripts (such as methods using multiple rounds of PCR, i.e., Jena et al.).

One of skill in the art would not arrive at Applicants' claimed invention based on the teachings of Jena et al. as Jena et al. neither teaches nor suggests a linearly amplified population of labeled cDNA obtained from fewer than 1000 cells for use in determining relative expression of at least two genes. Instead, the methods of Jena et al. require the use of two or more rounds of amplification. See section 2.8, where Jena et al. teaches two rounds of amplification and page 206, right column, first full paragraph, where Jena et al. teaches three rounds of amplification to visualize single molecule amplification. As discussed above, Applicants have determined that using two or more rounds of amplification results in non-linear amplification of the cDNA and has the effect of disproportionately amplifying abundant transcripts relative to rare transcripts. Thus, the methods used by Jena et al. would not result in linear amplification, as is required by the instant claims. Accordingly, Jena et al. fails to render the claimed invention obvious.

Lockhart et al. fails to cure the deficiencies of the primary reference. Lockhart et al. is directed to gene expression monitoring using high density arrays. Lockhart et al. neither teaches nor suggests a linearly amplified population of labeled cDNA obtained from fewer than 1000 cells for use in determining relative expression of at least two genes, as required by the instant claims. Accordingly, Applicants respectfully request that rejection of claims 1-3, 5 and 9-31 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

III. Claims 4, 6-8, 41, 45 and 46 Are Patentable Over Jena et al. in View of Lockhart et al. in Further View of Hampson et al.

At page 4, paragraph 2 of the instant Office Action, claims 4, 6-8, 41, 45 and 46 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Jena et al. in view of Lockhart et al., in further view of Hampson et al., U.S. Patent No. 6,066,457 for the reasons of record. Applicants respectfully traverse this rejection.

Applicants' claims are directed to novel methods of monitoring gene expression wherein RNA is obtained from a sample comprising fewer than 1000 cells or from a single cell, a first population of cDNA is generated from the RNA, the first population of cDNA is ***linearly amplified*** to produce a linearly amplified second population of cDNA, the linearly amplified second population of cDNA is labeled with a detectable label, and an array of probes is contacted with the linearly amplified population of labeled cDNA to determine relative expression of at least two genes.

As discussed above, the combination of Jena et al. and Lockhart et al. fails to teach or suggest Applicants' claimed invention. Hampson et al. fails to cure the deficiencies of the primary references. Hampson et al. neither teaches nor suggests a linearly amplified population of labeled cDNA obtained from fewer than 1000 cells for use in determining relative expression of at least two genes, as required by the instant claims. Thus, the combination of references fails to teach or suggest the claimed invention. Accordingly, Applicants respectfully request that the rejection of claims 4, 6-8, 41, 45 and 46 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

IV. Claims 32-44 Are Patentable Over Jena et al. in View of Lockhart et al. in Further View of Church et al.

At page 5, paragraph 6 of the instant Office Action, claims 32-44 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Jena et al. in view of Lockhart et al. in further view of Church et al., U.S. Patent No. 6,511,803 for the reasons of record. Applicants respectfully traverse this rejection.

Applicants' claims are directed to novel methods of monitoring gene expression wherein RNA is obtained from a sample comprising fewer than 1000 cells or from a single cell, a first population of cDNA is generated from the RNA, the first population of cDNA is ***linearly amplified*** to produce a linearly amplified second population of cDNA, the linearly amplified second population of cDNA is labeled with a detectable label, and an array of probes is contacted with the linearly amplified population of labeled cDNA to determine relative expression of at least two genes.

As discussed above, the combination of Jena et al. and Lockhart et al. fails to teach or suggest Applicants' claimed invention. Church et al. fails to cure the deficiencies of the primary references. Church et al. neither teaches nor suggests a linearly amplified population of labeled cDNA obtained from fewer than 1000 cells for use in determining relative expression of at least two genes, as required by the instant claims. Thus, the combination of references fails to teach or suggest the claimed invention.

The Examiner further asserts that in the response filed June 25, 2004, Applicants argued that while the method determines relative expression of at least two genes in fewer than 1000 cells, claims 32-40 and 42-44 do not recite such a limitation. Applicants respectfully disagree. In Applicants' response of June 25, 2004, Applicants stated the claims were directed to methods of determining gene expression wherein RNA is isolated from a sample comprising fewer than

1000 cells ***or from a single cell***...and wherein the ***relative expression*** of two or more genes can be determined. Independent claim 32 is directed to RNA from ***a first cell***, RNA from ***a second cell***, and identifying at least two genes that are ***differentially expressed***. Applicants respectfully submit that differential expression is a relative measurement of expression determined by comparing two or more values. Independent claim 39 is directed to a ***single cell*** population of RNA, and determining ***relative expression*** of at least two genes. Independent claims 40, 42 and 43 are directed to a ***single cell*** population of RNA, and determining ***relative expression*** of a plurality of genes. Accordingly, claims 32-40 and 42-44 are directed methods of determining relative expression of two or more genes wherein RNA is isolated from a sample comprising fewer than 1000 cells or from a single cell.

Accordingly, Applicants respectfully request that the rejection of claims 32-44 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

V. Conclusion

Having addressed all outstanding issues, Applicants respectfully request entry and consideration of the foregoing amendments and reconsideration and allowance of the case. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is requested to telephone the undersigned at the number below.

Respectfully submitted,

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